

# Protective Effect of the whole plant of *Mollugo pentaphylla* Linn. against Carbon Tetrachloride induced Hepatotoxicity in Rats

R. Valarmathi<sup>\*1</sup>, A.Rajendran<sup>2</sup>, V. Gopal<sup>3</sup>, R. Senthamarai<sup>1</sup>, S. Akilandeswari<sup>1</sup>  
and B.Srileka

<sup>\*1</sup>Periyar College of Pharmaceutical Sciences for Girls, Tiruchirappalli, Tamilnadu,India

<sup>2</sup>Department of Botany, Bharathiyar University, Coimbatore, Tamil Nadu,India

<sup>3</sup>College of Pharmacy Mother Theresa Post Graduate and Research Institute for Health Sciences, Puducherry,India

*\*Corres. Author: akipcog@yahoo.co.in*

**Abstract:** The present study was aimed at investigating the hepatoprotective activity of the alcoholic extract of *Molluga Pentaphylla lin.* (aizoaceae) against carbon tetrachloride induced hepatotoxicity in rats. Liver function was assessed by then determination of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGOT), alkaline phosphatase (ALP), acyl carrier protein (ACP), billurbin. Histopathological studies revealed that concurrent administration of the extract with carbon tetrachloride exhibited protective of the liver, which further evidenced its hepatoprotective activity. The result suggest that the use of alcoholic extract of *Mollugo pentaphylla.* exhibited significant protective from liver damage in CC<sub>14</sub> induced liver damage model. Phytochemical screening of this plant revealed the presence flavanoids, saponins, terpenoids and tannis.

**Key words:** *Mollugo pentaphylla* Linn., Carbon Tetrachloride induced Hepatotoxicity in Rats.

## Introduction

Liver is the most important organ, which plays a pivotal role in regularity various physiological process in the body. It has great capacity to detoxicate toxic substances and synthesis useful principles. Therefore damage to the liver inflicted by hepatotoxic agents is of grave consequences<sup>1</sup>. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages<sup>2</sup>.

Inspite of tremendous advances in modern medicine, there are no effective drug available the stimulate liver function, offer protection to the liver from damage or help to regenerate hepatic cells<sup>3,4</sup>.

In absence of reliable liver protective drugs in modern medicine, there exists a challenge for pharmaceutical scientists to explore the potential of

hepatoprotective activity in plants on the basis of traditional use<sup>5</sup>.

There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional system of medicine<sup>6</sup>. Historically plants have been used in folk medicine to treat various diseases and are rich natural sources of antioxidants. Many researchers have examined the effect of plants used traditionally by indigenous people to support liver function and treat diseases of the liver. In most cases, research has confirmed traditional experience by discovering the mechanism and mode of action of these plants. The present study was carried out to evaluate the hepatoprotective activity of the alcoholic extract of the whole plant of *Mollugo pentaphylla* against carbon tetrachloride induced hepatotoxicity in rats.

Carbon tetrachloride was the first toxin for which it was shown that the injury it produces is largely or entirely mediated by a free radical mechanism. Carbon tetrachloride is lipid soluble and hence cross cell membranes and gets distributed to all organs.

#### About plant

*Mollugo pentaphylla* (*Aizoaceae*), commonly known as *Mollugo stricta*, is a small genus of herbs distributed through out the warmer regions of the world extending into Europe and North America. In folk medicine, *Mollugo pentaphylla* has been used as a anti cancer, antitoxic while a decoction of the roots is used to treat eye diseases. Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases. The present work attempt to evaluate the flavonoid rich alcoholic extract of *Mollugo pentaphylla* using CCl<sub>4</sub> induced liver damage model in rats.

#### Material and Methods

##### Plant Material

The fresh whole plant of *Mollugo pentaphylla* were collected from Tirunelveli, Tamilnadu, India, in the month of June 2008 and were authenticated by comparing the herbarium specimens in the Department of Botany Central Siddha Research Unit, Tirunelveli. The voucher specimen of the plant has been preserved in herbarium of Periyar College of Pharmaceutical Sciences, Tiruchirappalli.

##### Preparation of extract

The dried whole plant was pulverized into coarse powder. The powder was sieved and extracted with alcohol and water by maceration process

separately. These extracts were concentrated under vacuum and used for further investigation.

##### Phytochemical Screening

Phytochemical analysis by the qualitative chemical tests was conducted for the detection of flavonoids, saponins, terpenoids and tannins.

##### Chemicals

All chemicals were of the high quality from BDH Chemicals and Sigma Aldrich Chemical Co. All other reagents were of analytical grade. All solutions were prepared in double-distilled deionised water.

##### Experimental Animals

Adult Wistar albino rats of either sex weighing between 150-200g were used for the study. They were kept under standard laboratory conditions and were fed with commercial rat pellets and drinking water *ad libitum*. The experiment was performed under the guidance of the Institutional Animal Ethics Committee. (Registration No. 265/ CPCSEA)

##### Acute toxicity studies

Wister albino rats (150-200g) maintained under standard laboratory condition was used. A total of fine animals were used for each extract which received a single oral dose (200mg/kg, body weight) of the extract. Animals were kept over night fasting prior to drug administration. After the administration of the extracts, food was withheld for further 3-4 hrs. Animals were observed individually once during the first 30min after dosing, periodically during the first 24hrs (with special attention during the first 4 h) and daily there after for a period of 14 days. Once daily cage side observation included changes in skin and fur, eyes and mucous membrane, and also respiratory rate, circulatory, autonomic and CNS changes<sup>7</sup>.

**Table 1: Hepatoprotective activity of *Mollugo pentaphylla* and silymarin on CCl<sub>4</sub> induced liver damage in rats**

Treatment	SGOT(U/I)	SGPT(U/I)	ALP(IU/I)	ACP(U/L)	Bilirubin
Control	97.3 ± 6.6	35.08 ± 2.2	15.92 ± 1.2	10.5 ± 0.98	0.39 ± 0.04
CCl <sub>4</sub> [0.5ml/kg]	186.7 ± 5.2	136.9 ± 7.6	98.3 ± 4.3	38.6 ± 2.2	0.89 ± 0.03
Alcohol Extract (200 mg/kg)	117.31 ± 5.2	64.31 ± 1.2	44.12 ± 1.5	19.4 ± 0.86	0.60 ± 0.02
Silymarian (25 mg /kg)	105.3 ± 3.5	49.4 ± 2.3	43.6 ± 1.7	16.5 ± 0.96	0.57 ± 0.02

Data are expressed as mean ± S.E.M., n=6  
p<0.01 Vs Control

### Selection of dose of the extract

LD<sub>50</sub> was determined as per OECD guidelines for fixing the dose for biological evaluation. The LD<sub>50</sub> of the extracts as per OECD guidelines falls under category 4 values with no signs of acute toxicity at doses of 2000 mg/kg. Hence the biological evaluation of the extracts was carried out at a dose of 200 mg/kg.b.w.

### Induction of experimental hepatotoxicity

Hepatotoxicity was induced in rats by injecting carbon tetrachloride intraperitoneally at a dose of 0.5ml/kg. b.w. for 7 consecutive days<sup>8</sup>.

### Hepatoprotective activity

Animals were divided into five groups, consisting of six animals each. Group 1 served as solvent control, which received normal saline (10 ml/kg, p.o). Group II received CCl<sub>4</sub> (0.5ml/kg, i.p) for 7 days. Group III received CCl<sub>4</sub> (0.5 ml/kg, i.p) and *Mollugo pentaphylla* extract (200 mg/kg, p.o.), Group IV received CCl<sub>4</sub> and alcoholic extract (200 mg/kg,p.o) and Group V received CCl<sub>4</sub> and silymarin (25mg/kg, p.o) simultaneously for 7days<sup>9</sup>.

### Assay of serum SGOT, SGPT, ACP, ALP, total bilirubin levels.

The collected blood was centrifuged at 2000 rpm for 15 min to separate the serum. The biochemical estimation was carried out using semi auto-analyser. The serum was then analysed for the biochemical parameters serum glutamate-oxaloacetate transaminase (SGOT), serumglutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), carrier protein (ACP) and total bilirubin.

### Histopathological studies

A portion of liver tissue in each group was preserved in 10% formaldehyde solution for histopathological studies. Haemotoxylin and eosin were used for staining and later the microscopic slides of the liver cells were photographed at a magnification of 100<sub>x</sub><sup>10</sup>.

Drug treatment (extract and standard) was started 5 days prior to CCl<sub>4</sub> administration and continued till the end of the experiment. After 48 hrs following CCl<sub>4</sub> administration, the rats were sacrificed using ether anaesthesia. Blood was collected by heart puncture and serum was separated by centrifugation. The liver was removed and a small piece was cut and fixed on 10% formalin for the histopathological studies.

### Statistical Analysis

Values were represented as mean + SEM. Data were analyzed by one way analysis of variance

(ANOVA) followed by Dunnett's test.  $p < 0.01$  was considered significant.

### Results

The Preliminary phytochemical screening of whole plant of *Mollugo pentaphylla* revealed the presence of flavonoids, saponin, terpenoids and tannis.

Rats treated with CCl<sub>4</sub> alone developed significant hepato - cellular damage as was evident from a significant ( $P < 0.01$ ) increase in the serum levels of SGOT, SGPT, ALP, ACP and bilirubin concentration, when compared with the controls.

Histopathological examination of the liver tissues provided supporting evidence for the results obtained in the biochemical analysis. Liver from rats challenged with CCl<sub>4</sub> alone showed multiple foci of hepato cellular necrosis appearing mainly around central vein area and central part of the lobule along with massive steatosis and foci of lipid loaded clean hepatocytes. Lobular foci by necrotic and steatotic hepatocytes were surrounded by histocytic lymphocytic infiltrate. In animals pretreated with the plant extract and subsequently given CCl<sub>4</sub> there was relatively well preserved cytoarchitecture around periportal tract area.

### Discussion

The liver performs many functions vital to the health of the organism. The liver is the target organ for toxic drug - induced lesions. The physiological response to injury results in a variety of lesions such as necrosis, cholestasis, steatosis, inflammation and fibrosis<sup>11</sup>. Carbon tetrachloride induced hepatotoxicity serves as an excellent model to study the molecular, cellular and morphological changes in the liver<sup>12</sup>.

CCl<sub>4</sub> is concentrated in the liver and it is the principle site of toxicity. CCl<sub>4</sub> is metabolized by the cytochrome P<sub>450</sub> system to give the trichloromethyl radical (CCl<sub>3</sub>). This radical combines directly with the biological molecular causing covalent modification as well as abstracting hydrogen from membrane lipids, promoting the chain reaction of lipid peroxidation. Peroxidation products inhibit protein synthesis and cause leakage of serum enzymes like AST, ALT and ALP and an elevation in the levels of thiobarbituric acid reactive substances and depletion of GSH and other anti oxidant enzymes, culminating in liver damage.

The hisopathological observation in CCl<sub>4</sub> - treated rats showed typical centrilobular hepatocytic steatosis (both macro and micro vesicular) and necrosis, limiting plate necrosis, apoptosis, especially

in the periportal hepatocytes and portal triaditis. This could be due to the formation of highly reactive free radicals because of oxidative stress caused by CCl<sub>4</sub>. Simulations administration of the extract along with CCl<sub>4</sub> prevented these effects. Thus histopathological studies revealed that concurrent administration of CCl<sub>4</sub> with the extracts exhibits protection of liver cells which further confirmed the above results.

Standard drug, Silymarin used in the present study is a well known hepatoprotective compound. The hepatoprotective properties of silymarin have also been confirmed in clinical studies<sup>13</sup>. Silymarin is a mixture of flavanolignans, primarily consisting of silybin, silydianin and silychristin<sup>14</sup>. It is reported to have marked protective effect on the plasma membrane of hepatocytes<sup>15</sup>.

### Conclusion

Taken as whole, although the effect of 200 mg alcoholic extract of *Mollugo pentaphylla* in preventing

CCl<sub>4</sub>- induced hepatotoxicity as reflected in the serum biochemical parameters and percentage of hepatocytes viability does not seem to be drastic and as effective as standard drug, Silymarin, it points to a favourable disposition toward recovery among all the parameters.

In conclusion, the results of this study clearly demonstrated that the plant extracts exhibited potent hepatoprotective activity against carbon tetrachloride-induced hepatic damage in rats. This may be due to its antioxidant and free radical scavenging properties. Further studies of needed to isolate the individual bioactive compounds with regard to the protective mechanism of the *Mollugo pentaphylla* against CCl<sub>4</sub>-induced liver injury.

### Acknowledgement

The authors are thankful to the Principal and Management, Periyar College of Pharmaceutical Sciences, Tiruchirapalli for providing laboratory and experimental facilities for this study.

### References

1. Sahani S. Evaluation of hepatoprotective efficacy of APCL-A poly herbal formulation in vivo in rats. *Indian drugs*. 1999;36;628-631.
2. Recknagel RO, A new direction in the study of carbon tetrachloride hepatotoxicity. *Life Sciences*, 1983; 33,401 – 408.
3. Chattopadhyay RR, Possible Mechanism of Hepatoprotective activity of *Azadirachta indica* leaf extract. *Journal of Ethanopharmacology*, 2003; 89; 217-219.
4. Shanmuga Sundaram P and Venkataraman S Hepatoprotective and anti oxidant effects of *Hygrophila Auriculata* Heine – Root extract. *J. Ethano pharmacology*.2006;104:124-128.
5. Witte I, Berlin J, Wray V, Schubert W, Kohl W, Hofle G and Hammer J. mono and di terpins from cell culture or Thuja Occidenalis. *Planta medica* 1983;49:216-221.
6. Malhotra S, singh AP, Hepatoprotective Natural Products ( online). (Cited 2006 Nov 11), available from: [URL:http://www.siv.edu/eb/leaflets/hepato.htm](http://www.siv.edu/eb/leaflets/hepato.htm).
7. OECD. Acute oral toxicity acute oral toxic class method. Guideline 423, adopted 23.03.1996. In eleventh Addendum to the OECD Guidelines for the testing of chemicals, Paris, Organization for economic co-operation and development, 2000.
8. Uma maheswari M, Rao GM, Anti hepatotoxic effect of grape seed oil, *Indian J pharmacol*, 2005, 37, 179-182.
9. Uma maheswari M, Asok kumar K, Ravi TK etal: *Drug lines*, 2007, 9, 14-18.
10. Prophet E.B, Mills B, Arrington J.B, Sobin L.H. Laboratory method in Histotechnology, Washington DC, Armed Forces Institute of Pathology, 1992.
11. Berine R.M, Levy M.N, Principles of Physiology, London, Wolf Publishing Limited, 1990,391-393.
12. Clawson GA, Mechanism of Carbon tetrachloride hepatotoxicity, *ImmunoPathol Res*,1989,8,104-112.
13. Ferenci P, Gragosics B, Dittrich H, Benda L, Loches H, Meryn S, Base W, Schneider B, *Journal of Hepatology*, 1989, 105-113.
14. Grange LL, Wang M, Watkins R, Ortiz D, Sanchez ME, Konst J, Lee C, Reyes E. *Journal of Ethanopharmacology*, 1999, 65, 53 – 61.
15. Visen PKS, Shukla B, Patnaik GK, dhawan BN. *Journal of Ethanopharmacology*, 1993, 40, 131- 136.

\*\*\*\*\*